

Exposure to Peroxisome Proliferators: Reassessment of the Potential Carcinogenic Hazard

Melnick (1) recently suggested that because peroxisome proliferation is not established as an obligatory step in the carcinogenicity of peroxisome proliferators (PPs), the proposal that the peroxisome proliferator di(2-ethylhexyl)phthalate (DEHP) poses no carcinogenic risk to humans (2) due to species differences in peroxisome proliferation should be viewed as an unvalidated hypothesis (1). In this context, Melnick (1) raised the recent downgrading by the International Agency for Research on Cancer (IARC) of DEHP (3) to "not classifiable as to its risk to humans (group 3)" based on their conclusion that it produces rodent liver tumors by a mechanism involving peroxisome proliferation, which they judged to be not relevant to humans (3). As illustrated by Melnick (1), there is a large body of data to correlate the phenomenon of rodent liver peroxisome proliferation with rodent liver cancer but "published studies have not established peroxisome proliferation per se as an obligatory pathway on the carcinogenicity of DEHP" (1). This focuses attention on the need, as also suggested by O'Brien et al. (4), for a fundamental review of how PPs induce liver cancer in rodents and the relevance of these rodent tumors for humans.

There are two distinct usage patterns for PPs: as drugs such as clofibrate for the treatment of hypolipidemia (5) and in non-clinical applications such as the plasticizer DEHP. Most PPs are carcinogenic to the rodent liver, and the task of assessing their human carcinogenic potential has fallen to different regulatory agencies, depending on the primary usage pattern of the particular PP in question. There seems to be little regulatory concern regarding the safety of the clinical PPs, yet continuing uncertainty regarding the safety of the nonpharmaceutical PPs. This presents an untenable situation that we suggest is unjustified.

Hypolipidemic fibrates such as clofibrate and gemfibrozil have been used extensively over the past 20 years to treat cardiovascular disease and are enjoying a revival due to recent reconfirmation of efficacy (6). However, by 1980 several of these agents had become associated with a rodent-specific response known as hepatic peroxisome proliferation (7), a property shared by a number of nonpharmaceutical chemicals (8). Additionally, a link between rodent liver peroxisome proliferation and an increased risk of rodent liver carcinogenesis was emerging (7). Nonetheless, clinical side

effects of the fibrate PPs are rare, and analyses of causes of death during treatment show no evidence of an adverse effect and no evidence of an increase in malignant disease compared to the normal population (5). Specifically, the carcinogenic risk to humans of gemfibrozil and clofibrate has been formally evaluated by IARC (9,10), and in the case of clofibrate, for which most clinical data exist, IARC (9) concluded that "the mechanism of liver carcinogenesis in clofibrate-treated rats would not be operative in humans." This conclusion was based on the observation that clofibrate causes peroxisome proliferation and cell proliferation in rodent but not human hepatocytes and on the results of extensive epidemiologic studies, particularly the World Health Organization trial on clofibrate that included 208,000 man-years of observation (11,12). Further, a meta-analysis (13) of the results from six clinical trials on clofibrate also found no excess cancer mortality (9).

It therefore appears that there are no remaining concerns about the human carcinogenic potential of the clinical PPs and that the rodent liver effects have been set aside as probable laboratory curiosities. However, this is not true for the nonpharmaceutical PPs; several regulatory agencies continue to be concerned about their carcinogenic potential to the human liver. The unease of these agencies is due to their belief that in the absence of a definitive mechanism of PP-induced rodent liver carcinogenesis, it is not possible to make a clear statement on the human safety of these chemicals. Nonetheless, there are now several strong lines of evidence that PP-induced rodent liver carcinogenesis is not relevant to humans, which supports the conclusion drawn by IARC for the clinically used PPs and the recent IARC decision to downgrade DEHP from group 2B (possibly carcinogenic to humans) to group 3 (3). These lines of evidence are as follows:

- Direct genetic toxicity has been eliminated as a common mechanism of carcinogenic action for PPs in general (14). Thus, rodent hepatocarcinogenicity must occur via a nongenotoxic mechanism that correlates with peroxisome proliferation, although, as pointed out by Melnick (1), the hepatocarcinogenicity is unlikely to be caused by peroxisome proliferation per se, as initially suggested (15).
- There are marked species differences in the induction of peroxisomes, with human hepatocytes being resistant (8,16,17). These data provide evidence that the phenomenon of PP-induced peroxisome proliferation is rodent specific.
- PPs suppress rodent hepatocyte apoptosis

(18–20) and induce rodent hepatocyte replication (8). This duality of effects provides a plausible mode of rodent carcinogenic action based on liver growth perturbation (21,22). As well as being resistant to peroxisome proliferation, human hepatocytes are also resistant to PP-mediated induction of replication and suppression of apoptosis (8,16,17).

Whatever the precise mechanism by which PPs induce rodent liver cancer, rodent liver peroxisome proliferation, induction of the peroxisomal gene acyl CoA oxidase (*ACO*) (23), hypertrophy (24), and carcinogenicity (25) are all mediated through activation of the peroxisome proliferator-activated receptor (*PPARα*). This is illustrated dramatically by the absence of all of these responses in *PPARα* knockout mice treated with the PPs DEHP or Wyeth-14,643 (24–26).

Although human liver expresses around 10-fold less *PPARα* mRNA than the rodent liver (27,28), evidence suggests that the hypolipidemic effects of the fibrate drugs in humans are also mediated by activation of *PPARα*, leading to regulation of the apolipoprotein (*Apo*) genes such as *ApoA1* (29). Thus, *PPARα* levels in human liver may be sufficient to mediate PP-induced hypolipidemia, but insufficient to activate the gene battery associated with rodent peroxisome proliferation and cancer (30). In addition to these quantitative data, there are species differences in the molecular sequence of the *PPARα* response elements (*PPREs*) located upstream of genes associated with rodent peroxisome proliferation such as *ACO*. In the rat, *ACO* responds to PPs via a functional *PPRE*, whereas the equivalent gene in humans does not (31,32). Thus, the human *ACO* gene does not respond to PPs even in the presence of excess *PPARα* (31–33). Similarly, recent data have shown that *PPARα* cannot induce the battery of peroxisome proliferation-associated genes in human hepatoma cells (33,34). Conversely, the human *ApoA1* gene is responsive to fibrate PPs, whereas the equivalent rat gene is not (35). Such findings isolate the human hypolipidaemic effects of PPs from the rodent cancer effects.

Although the precise mechanism of the carcinogenic action of PPs in the rodent liver remains to be determined, all of the phenomena associated with this response of rodent hepatocytes (peroxisome proliferation, *ACO* gene expression, induction of cell proliferation, and the suppression of apoptosis) are absent in human hepatocytes. This body of data provides a plausible mode of carcinogenic action for the rodent liver,

which, when coupled with the clinical epidemiology showing an absence of a human cancer risk, provides substantial weight of evidence that the PP class of nongenotoxic rodent hepatocarcinogens does not pose a potential cancer hazard to the human liver.

Ruth Roberts
Graeme Moffat
John Ashby

Syngenta CTL
Alderley Park, United Kingdom
E-mail: ruth.roberts@syngenta.com

REFERENCES AND NOTES

- Melnick RL. Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? *Environ Health Perspect* 109:437–442 (2001).
- Doull J, Cattley R, Elcombe C, Lake B, Swenberg J, Wilkinson C, Williams G, van Gamert M. A cancer risk assessment of DEHP: application of the new US EPA risk assessment guidelines. *Regul Pharmacol Toxicol* 29:327–357 (1999).
- IARC. On the Evaluation of Carcinogenic Risk to Humans. Some Industrial Chemicals. IARC Monogr Eval Carcinog Risk Chem Hum 77 (2000).
- O'Brien M, Twaroski T, Cunningham M, Glauert H, Spear B. Effects of peroxisome proliferators on antioxidant enzymes and antioxidant vitamins in rats and hamsters. *Toxicol Sci* 60:271–278 (2001).
- The coronary drug project research group. Clofibrate and niacin in coronary heart disease. *JAMA* 231:360–380 (1975).
- Milonis H, Elisaf M, Mikhailidis D. Treatment of dyslipidaemias in patients with established vascular disease: a revival of the fibrates. *Cur Med Res Opin* 16:21–32 (2000).
- Reddy JK, Azarnoff DL, Hignite CE. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283:397–398 (1980).
- Ashby J, Brady A, Elcombe CR, Elliot BM, Ishmael J, Odum J, Tugwood JD, Kettle S, Purchase IFH. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum Exp Toxicol* 13:S1–S117 (1994).
- IARC. Clofibrate. IARC Monogr Eval Carcinog Risk Hum. 66:391–426 (1996).
- IARC. Gemfibrozil. IARC Monogr Eval Carcinog Risk Hum. 66:427–444 (1996).
- WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: mortality follow-up. Report of the Committee of Principal Investigators. *Lancet* 2:379–385 (1980).
- WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. Report of the Committee of Principal Investigators. *Lancet* 2:600–604 (1984).
- Law M, Thompson S, Wald N. Assessing possible hazards of reducing serum cholesterol. *Br J Med* 308:373–379 (1994).
- Galloway SM, Johnson TE, Armstrong MJ, Ashby J. The genetic toxicity of the peroxisome proliferator class of rodent hepatocarcinogen. *Mutat Res* 448:153–158 (2000).
- Reddy JK, Rao MS. Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogenesis. *Mutat Res* 214:63–68 (1989).
- Hasmall S, James N, Macdonald N, Soames A, Roberts R. Species differences in response to diethylhexylphthalate (DEHP): suppression of apoptosis, induction of DNA synthesis and PPAR alpha-mediated gene expression. *Arch Toxicol* 74:85–91 (2000).
- Williams GM, Perrone C. Mechanism-based risk assessment of peroxisome proliferating rodent hepatocarcinogens. In: *Peroxisomes: Biology and Role in Toxicology and Disease*, Vol 804 (Reddy JK, ed). New York:New York Academy of Sciences, 1995:554–572.
- Bursch B, Lauer B, Timmerman-Trosienier I, Barthel G, Schuppler J, Schulte-Hermann R. Controlled death (apoptosis) of normal and preneoplastic cells in rat liver following withdrawal of tumor promoters. *Carcinogenesis* 5:453–458 (1984).
- Bayly AC, Roberts RA, Dive C. Suppression of liver cell apoptosis in vitro by the non-genotoxic hepatocarcinogen and peroxisome proliferator, nafenopin. *J Cell Biol* 125:197–203 (1994).
- Christensen J, Gonzalez A, Cattley R, Goldsworthy T. Regulation of apoptosis in mouse hepatocytes and alteration of apoptosis by nongenotoxic carcinogens. *Cell Growth Differ* 9:815–825 (1998).
- Roberts R. Peroxisome proliferators: mechanisms of adverse rodent effects and molecular basis for species differences. *Arch Toxicol* 73:413–418 (1999).
- Vanden Heuvel J. Peroxisome proliferator-activated receptors (PPARs) and carcinogenesis. *Toxicol Sci* 47:1–8 (1999).
- Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *EMBO J* 11:433–439 (1992).
- Lee SS-T, Pineau T, Drago J, Lee EJ, Owens JO, Kretz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 15:3012–3022 (1995).
- Peters JM, Cattley RC, Gonzalez FJ. Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator, Wy-14,643. *Carcinogenesis* 18:2029–2033 (1997).
- Ward J, Peters J, Perella C, Gonzalez F. Receptor and nonreceptor-mediated organ specific toxicity of DEHP in PPAR alpha null mice. *Toxicol Pathol* 26:240–246 (1998).
- Tugwood JD, Holden PR, James NH, Prince RA, Roberts RA. A peroxisome proliferator-activated receptor-alpha (PPARalpha) cDNA cloned from guinea-pig liver encodes a protein with similar properties to the mouse PPARalpha: implications for species differences in response to peroxisome proliferators. *Arch Toxicol* 72:169–177 (1998).
- Palmer CNA, Hsu M-H, Griffin KJ, Raucy JL, Johnson EF. Peroxisome proliferator activated receptor-alpha expression in human liver. *Mol Pharmacol* 53:14–22 (1998).
- Auwerx J. Regulation of gene expression by fatty acids and fibric acid derivatives: an integrative role for PPARs. *Horm Res* 38:269–277 (1992).
- Gonzalez F. The role of proxisome proliferator activated receptor alpha in peroxisome proliferation, physiological homeostasis and chemical carcinogenesis. In: *Dietary Fat and Cancer: Genetic and Molecular Interactions* (American Institute for Cancer Research, ed). New York:Plenum Press, 1997:109–125.
- Woodyatt N, Lambe K, Myers K, Tugwood J, Roberts R. The peroxisome proliferator (PP) response element (PPRE) upstream of the human acyl CoA oxidase gene is inactive in a sample human population: significance for species differences in response to PPs. *Carcinogenesis* 20:369–375 (1999).
- Lawrence J, Zhou G, Li Y, Chen S, Umbenhauer D, Moller D, DeLuca G. Species differences in response to peroxisome proliferators: increasing human PPAR alpha expression levels in human cells is not sufficient for fatty acyl CoA oxidase responsiveness. *Toxicol Sci* 54:322 (2000).
- Hsu M-H, Savas U, Griffin KJ, Johnson EF. Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor in HepG2 cells. *J Biol Chem* 276:27950–27958 (2001). Available: <http://www.jbc.org/cgi/content/full/276/30/27950> [cited 29 August 2001].
- Lawrence J, Li Y, Chen S, DeLuca J, Berger J, Umbenhauer D, Moller D, Zhou G. Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) α : PPAR α fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expression. *J Biol Chem* 276:31521–31527 (2001). Available: <http://www.jbc.org/cgi/content/full/276/34/31521> [cited 29 August 2001].
- Vu-Dac N, Chopin-Delannoy S, Gervois P, Bonnelye E, Martin G, Fruchart J-C, Laudet V, Staels B. The nuclear receptors PPAR alpha and RevErb alpha mediate the species specific regulation of apolipoprotein A1 expression by fibrates. *J Biol Chem* 273:25713–25722 (1998).

Peroxisome Proliferators: Response

The comments from Roberts et al. regarding my commentary (1) on whether human and mechanistic data provide a sufficient rationale to dismiss DEHP cancer risks because of species differences in peroxisome proliferation reveal both agreements and disagreements on this issue. We agree that the animal carcinogenicity data are irrefutable and, as written by Roberts et al., we agree that “the hepatocarcinogenicity [of peroxisome proliferators (PPs)] is unlikely to be caused by peroxisome proliferation per se.” In addition, we agree that the mechanisms of carcinogenic action of DEHP and other PPs remain to be determined.

Where we differ is on our views of what constitutes sufficient evidence to dismiss positive animal cancer data as being indicative of possible human cancer risk, as well as on interpretations of available data. From a public health perspective, it is important to reexamine the basis on which Roberts et al. conclude that rodent liver carcinogenesis induced by PPs “is not relevant to humans” while acknowledging that the mechanisms of carcinogenesis of PPs have not been established.

With respect to available human data, Roberts et al. refer to clinical studies on clofibrate and gemfibrozil in male subjects as providing evidence of “an absence of a human cancer risk” for fibrate PPs. In a previous review of these data, Ashby et al. (2) noted a small increase in basal cell carcinomas of the skin in gemfibrozil-treated patients, but concluded that the epidemiologic studies on hypolipidemic drugs “are of limited value only, because of the short time periods involved.” The World Health Organization trial on the prevention of ischemic heart disease by clofibrate was last updated in 1982 and included 13 years of observation, 5 years during the treatment period plus 8 years of follow-up (3). That study revealed an excess of deaths for non-malignant diseases of the liver, gall bladder, and intestines in the clofibrate-treated group compared to controls. Because the

latency period for clinical manifestation of cancer may be 20 years or more postexposure, the current data are insufficient to permit a definitive conclusion on the presence or absence of a causal association between exposure to fibrate lipid-lowering drugs and human cancer (4). It is not clear why Roberts et al. claim that "there are no remaining concerns about the human carcinogenic potential of the clinical PPs" inasmuch as a previous review from their laboratory of the same epidemiologic studies found these data to be of limited value due to short study durations (2), and there are no available studies on female cancer risk. Furthermore, in contrast to the view given by Roberts et al., the *Physicians' Desk Reference* (5) warns

of the tumorigenicity of clofibrate [and of gemfibrozil (6)] in rodents and the possible increased risk of malignancy associated with clofibrate in the human.

For DEHP, no epidemiologic studies have been reported.

Roberts et al. also conclude that PPs would "not pose a potential cancer hazard to the human liver." However, as I noted previously (1), it might not be appropriate to expect exact site correspondence for effects of PPs in rodents and humans because of species differences in tissue expression of PPARs. For example, the demonstration of a functional PPAR in human breast cancer cell lines (7) and the finding of enhanced cell proliferation by DEHP in human breast cancer cells (8) indicate a possible breast cancer risk. Furthermore, liver is not the only target organ of tumor induction by hepatic PPs. Several PPs induce tumors of the testis and pancreas in laboratory animals, and tumor induction at these sites occurs without induction of peroxisomes in these affected organs (9).

Roberts et al. claim that available data provide "a plausible mode of carcinogenic action" for PPs, which is based on induction of hepatocyte proliferation and suppression of apoptosis. The latter effects are reported to be absent in cultured human hepatocytes. However, as noted in the *Physicians' Desk Reference* (5,6),

changes in peroxisome morphology and numbers have been observed in humans after treatment with several members of the fibrate class, including clofibrate, when liver biopsies were compared before and after treatment in the same individual.

Several additional issues influence the plausibility of the mode of action for liver carcinogenicity claimed by Roberts et al.:

- Their hypothesis has not been challenged or demonstrated experimentally. For example, no studies have established time- and

dose-dependent associations between tumor induction and hepatocyte proliferation and suppression of apoptosis in laboratory animals treated with various PPs.

- It is important to recognize that procedures used to harvest hepatocytes from human livers are usually very different from those used to culture rodent hepatocytes. To evaluate possible functional loss during the time after death until culturing of human hepatocytes, I stressed the critical need to include demonstration of apolipoprotein A-II (ApoA-II) induction in studies on the responsiveness of human hepatocytes to PPs (1). ApoA-II mRNA has been shown to be induced in human hepatocytes via PPAR activation (10). Thus, measurements of ApoA-II induction provide a necessary control to decipher the absence of an effect as being due to the lack of responsiveness to PPs rather than lack of transcriptional function in human cell cultures.
- Because tumor induction by PPs requires long-term exposure, the mechanistic steps in this process most likely involve sustained rather than transient effects. In the case of DEHP and several other PPs, hepatocyte proliferation is not a sustained response with continued exposure (11). Further, cell culture studies are usually performed over a period of a few days and therefore cannot distinguish a sustained response from a transient effect.
- Mechanistic studies in normal human hepatocytes may be focusing on the wrong cell population. Cattley et al. (12) provided data that indicate that the mechanism of liver tumor induction by PPs may be more relevant to changes that are induced in preneoplastic foci rather than in normal hepatocytes.
- Several studies demonstrate that DEHP induces biological effects independent of peroxisome proliferation [e.g., morphologic cell transformation and decreased levels of gap junction intercellular communication (13)]. Roberts et al. ignore the possibility that some of these responses may also contribute to the carcinogenic process. Roberts et al. cite the 11-month study of Wy14,643 in PPAR α knockout mice (14) as evidence that the carcinogenicity of PPs are mediated by PPAR α . However, as I noted previously (1), unlike this mouse model, humans do not lack a functional PPAR α , and an 11-month study is not adequate to detect late-developing tumors that might arise by mechanisms independent of PPAR α .
- Roberts et al. cite the finding of an inactive PP response element upstream of the acyl CoA oxidase gene in a sample human population (15) as additional evidence for why humans may be less responsive than rodents to PPs. However, induction of acyl

CoA oxidase is not relevant to the suggested carcinogenic mode of action for DEHP and other PPs. Obviously, further work is needed to characterize the expression of all genes that may be affected by PPs in diverse populations.

The inappropriate dismissal of positive animal cancer findings in assessments of human risk could have serious health consequences. Protection of public health requires rigorous testing and validation of mechanistic hypotheses rather than reliance on assertions of plausibility.

Ronald Melnick

National Institute of Environmental
Health Sciences
Research Triangle Park, NC
E-mail: melnickr@niehs.nih.gov

REFERENCES AND NOTES

1. Melnick RL. Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? *Environ Health Perspect* 109:437–442 (2001).
2. Ashby J, Brady A, Elcombe CR, Elliott BM, Ishmael J, Odum J, Tugwood JD, Kettle S, Purchase IF. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum Exp Toxicol* 13:S1–117 (1994).
3. WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. Report of the Committee of Principal Investigators. *Lancet* 2:600–604 (1984).
4. Newman TB, Hulley SB. Carcinogenicity of lipid-lowering drugs. *JAMA* 275:55–60 (1996).
5. Atromid-S. In: *Physicians' Desk Reference*. 55th ed. Montvale, NJ:Medical Economics Data, 2001:3349–3350.
6. Lopid. In: *Physicians' Desk Reference*. 55th ed. Montvale, NJ:Medical Economics Data, 2001:2453–2456.
7. Kilgore MW, Tate PL, Rai S, Sengoku E, Price TM. MCF-7 and T47D human breast cancer cells contain a functional peroxisomal response. *Mol Cell Endocrinol* 129:229–235 (1997).
8. Blom A, Ekman E, Johannisson A, Norrgren L, Pesonen M. Effects of xenoestrogenic environmental pollutants on the proliferation of a human breast cancer cell line (MCF-7). *Arch Environ Contam Toxicol* 34:306–310 (1998).
9. Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci* 60:44–55 (2001).
10. Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B, Auwerx J. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J Clin Invest* 96:741–750 (1995).
11. Melnick RL, Huff J. Liver carcinogenesis is not a predicted outcome of chemically induced hepatocyte proliferation. *Toxicol Ind Health* 9:415–438 (1993).
12. Cattley RC, Marsman DS, Popp JA. Age-related susceptibility to the carcinogenic effect of the peroxisome proliferator Wy-14,643 in rat liver. *Carcinogenesis* 12:469–473 (1991).
13. Mikalsen SO, Sanner T. Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. *Carcinogenesis* 14:251–257 (1993).
14. Peters JM, Cattley RC, Gonzalez FJ. Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* 18:2029–2233 (1997).
15. Woodyatt NJ, Lambe KG, Myers KA, Tugwood JD, Roberts RA. The peroxisome proliferator (PP) response element upstream of the human acyl CoA oxidase gene is inactive among a sample human population: significance for species differences in response to PPs. *Carcinogenesis* 20:369–372 (1999).

The International Tissue and Tumor Repository for Chronic Arseniasis at the Armed Forces Institute of Pathology

The public health concern for environmental exposure to arsenic has been widely recognized for decades. However, recent human activities have resulted in even greater arsenic exposures and the potential increase for chronic arsenic poisoning on a worldwide basis. This is especially the case in China, Taiwan, Thailand, Mexico, Chile, India, and Bangladesh. The sources of arsenic exposure vary from burning arsenic-rich coal (China) (1) and mining activities (Malaysia, Japan) to the ingestion of arsenic-contaminated drinking water (Taiwan, Inner Mongolia, China) (2). The ground-water arsenic contamination in Bangladesh and the West Bengal Delta of India has received the greatest international attention due to the large number of people exposed and the high prevalence of arsenic-induced diseases (3). Recent estimates suggest that in West Bengal as many as 20–30 million people are at risk from drinking arsenic-contaminated water obtained from thousands of tube wells that appear to be contaminated with naturally occurring arsenic.

Although the health effects associated with chronic arsenic exposure have been reasonably well characterized in those areas around the world with high arsenic levels in their drinking water, the association of adverse health effects with arsenic exposure in the United States is less clear. This is primarily due to the lower exposure levels in the great majority of U.S. drinking water supplies and the lack of research studies that look for health effects in arsenic-exposed persons.

Cancer is a well-established arsenic-related disease, although the cancer risk at low level exposure is unclear. Equally unclear is whether low-level arsenic exposures may play a role in the incidence in non-cancer health problems in the United States such as immune suppression and cardiovascular disease. This uncertainty in dose effects at low levels has resulted in hotly debated differences in opinion as to the need for stricter government regulation of arsenic in drinking water. Recently, the National Research Council studied the issue and determined that the current

drinking water standard for arsenic is too high and recommended that it be lowered (4). The U.S. Environmental Protection Agency (U.S. EPA), in fact, has proposed lowering the allowable drinking water standard from 50 µg/L to 10 µg/L. The drinking water industry, however, opposes changing the standard on the basis that more information is needed as to how arsenic causes cancer and other health effects, and whether these mechanisms operating at high dose levels also operate at low levels as well.

It is true that the mechanisms by which arsenic may induce adverse health effects are largely unknown. There are two major reasons for this. One is that the sophisticated molecular probes needed to study cellular and biochemical mechanisms have only recently become available, whereas most of the toxicity studies with arsenic were conducted many years before their availability. A second, equally important reason is that properly fixed human tissues from arsenic-exposed persons are not available for such sophisticated research studies. The dilemma we face is that now we have the research tools (with even more sophisticated methods on the horizon), but we do not have the human tissues to study.

The Armed Forces Institute of Pathology (AFIP) is participating in an international research effort aimed at the development of an International Tissue and Tumor Repository for Chronic Arseniasis in Humans (ITTRCA). This effort is supported by four other federal agencies, including the U.S. EPA, the National Cancer Institute, the National Institute of Environmental Health Sciences, and the U.S. Geological Survey. The main objective of the ITTRCA is to obtain and archive tissues from persons known to or suspected of having been exposed to arsenic (environmental or occupational) and exhibiting adverse health effects. A component of this repository is to provide a mechanism by which the underlying pathology and morphology of arsenic-induced lesions can be described (5). The major thrust of the ITTRCA is to facilitate the formulation of a standardized system of nomenclature for the study of skin lesions and other arsenic-induced changes in tissues, to foster the use of archival materials for the development of international collaborative projects on arsenic health effects, to

facilitate the use and accessibility of archival materials associated with arsenic exposure, and to develop interlaboratory trials for the analysis and speciation of arsenic in biological tissues. Another unique component of the ITTRCA is the link to a repository of sources (coal, ores, soil, water) for arsenic exposures organized and maintained by the U.S. Geological Survey.

We request that pathologists, clinicians, epidemiologists, toxicologists, and public health professionals in the United States and throughout the world participate in this exciting international project. Please contact us for further information about the ITTRCA and the methods for collection and shipment of tissues.

Jose A. Centeno
Florabel G. Mullick

U.S. Armed Forces Institute of Pathology
Washington, D.C.

E-mail: centeno@afip.osd.mil
Telephone: (202) 782-2839

Herman Gibb

U.S. EPA, National Center for
Environmental Assessment
Washington, D.C.

David Longfellow

National Cancer Institute
Bethesda, Maryland

Claudia Thompson

National Institute of Environmental
Health Sciences
Research Triangle Park, North Carolina

REFERENCES AND NOTES

1. Finkelman RB, Belkin HE, Zheng BS. Health impacts of domestic coal use in China. *Proc Natl Acad Sci USA* 96:3272–3431 (1999).
2. Chen CJ, Hsu LI, Tseng CH, Hsueh YM, Chiou HY. Emerging epidemics of arseniasis in Asia. In: *Arsenic Exposure and Health Effects* (Chappell WR, Abernathy CO, Calderon RL, eds). New York: Elsevier Science Ltd, 1999;113–121.
3. Guha Mazumder DN, De BK, Santra A, Dasgupta J, Ghosh N, Roy BK, Ghoshal UC, Saha J, Chatterjee A, Dutta S, et al. Chronic arsenic toxicity: epidemiology, natural history and treatment. In: *Arsenic Exposure and Health Effects* (Chappell WR, Abernathy CO, Calderon RL, eds). New York: Elsevier Science Ltd, 1999;335–347.
4. National Research Council. *Arsenic in Drinking Water*. Washington DC:National Academic Press, 1999.
5. Centeno JA, Martinez L, Ladich ER, Page NP, Mullick FG, Ishak KG, Zheng BS, Gibb H, Thompson C, Longfellow D. *Arsenic-Induced Lesions*. Washington, DC:Armed Forces Institute of Pathology, 2000.